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Entoloma conferendum, Hygrocybe coccineocrenata, and *Hypholoma ericaeum* new to Montenegro

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Abstract—As a result of the first research on macrofungi on peat moss habitats in Montenegro, three species new to the Montenegrin mycobiota are presented and described: *Entoloma conferendum*, *Hygrocybe coccineocrenata*, and *Hypholoma ericaeum*. The descriptions are accompanied by photographs of the basidiomata on site and some microscopic characters. Additionally, the second Montenegrin record of *Bovista paludosa* is presented and described, for the first time with locality and habitat data. Species identifications were checked by comparing ITS rDNA sequences from samples with the sequences in GenBank.

Key words—*Agaricales, Basidiomycota*, biodiversity, taxonomy

Introduction

Sphagnum (peat moss) is a genus with several hundred species that live in wet habitats and can store water in their cells. The most important habitats are peat bogs, where due to constant water availability and oxygen reduction, peat sediments form by the accumulation of the partially decomposed plant remnants. Peat bogs are characterised by acidic soils, low nutrient content,

and dominance of peat mosses. These habitats, which form in humid and cold climates, are widely distributed in northern parts of Asia, Europe, and North America. However, despite a still favourable climate, natural peatlands are vanishing from northern Europe due to human activities such as peat extraction, drainage, and land development. In the western Balkans, primarily due to unfavourable climate conditions, peat bogs are exceptionally rare and relict habitats, occupying only small highly isolated areas. They are particularly sensitive to climate changes, which can cause them to dry out and become overgrown by other vegetation types. Therefore, peat bogs are one of the most endangered ecosystems in Montenegro. Some macrofungal species live exclusively in this type of habitat, but a certain number of fungi with a wider habitat tolerance can also be found in peat bogs. Peat mosses can also live in wet, acidic habitats without forming peat, mostly in coniferous forests.

Due to the small number of researchers, Montenegrin fungi, including agarics, are rather poorly explored. Kasom (2013) lists 839 macro-basidiomycete species for Montenegro, 444 of which belong to the *Agaricales*. Bubák (1915), who conducted the first mycological research on Montenegrin peat bogs in 1904, reported several microfungi from the Barno jezero peat bog (Mt. Durmitor). However, until our research, no macrofungi had been reported in peat bogs of Montenegro. During the summer and autumn of 2017 and 2018 we surveyed macrofungi of mountain peat bogs and peat moss carpets on Mt. Sinjajevina, Mt. Durmitor, and Mt. Hajla. We describe three *Agaricales* species new to Montenegrin mycobiota, as well as one previously reported rare species with the first data on its locality and habitat in Montenegro.

Materials & methods

Collected basidiomata were photographed on the site, described, preserved by drying and deposited in the mycological collections of Natural History Museum of Montenegro, Podgorica (NHMP) and/or Croatian National Fungarium in Zagreb (CNF).

The morphological descriptions are entirely based on the collected material. Microscopic features are described from dried material mounted in 2.5% potassium hydroxide (KOH) and observed with a light microscope under magnification up to 1500×. Basidiospore measurements were made by Motic Images Plus 2.0 software on 30 randomly chosen mature basidiospores from calibrated digital images. Arithmetic means of basidiospore length and width are shown in italic font in the centre of the measurements. The length/width ratio of all measured basidiospores is given as the "Q" value (min. – av. – max.).

Genomic DNA was extracted from dried specimens using DNeasy Plant Mini kit (Qiagen) according to the manufacturer's instructions. PCR primers ITS1F and

ITS4 (Gardes & Bruns 1993) were used for ITS region amplification and sequencing purposes. The ITS rDNA regions were amplified in a thermocycler (Eppendorf) with a final volume of 25 μL containing approximately 50 ng DNA template, 0.2 μM of each primer, 0.2 mM dNTP, 1.5 mM $MgCl_2$, 1× buffer, and 1 unit of Taq polymerase (Invitrogen). The cycling protocol was: 94 °C for 5 min, 30 cycles (94 °C for 45, 60 °C for 45 s, 72 °C for 45 s), followed by a final extension of 72 °C for 10 min. The PCR products were resolved on a 1% agarose gel stained with ethidium bromide and viewed under ultraviolet light. PCR products were purified and sequenced by Macrogen Inc. (The Netherlands). Sequences were aligned in both directions and edited using Sequencher v.4.1.4 and submitted to GenBank.

Taxonomy

Hygrocybe coccineocrenata (P.D. Orton) M.M. Moser,
Röhrlinge & Blätterpilze, 3. Aufl.: 68 (1967). Fig. 1 a, b

Pileus 6–23 mm broad, convex at first, later applanate to concave with a depressed centre and mostly inflexed and somewhat crenate margin, scarlet red, orange red or reddish orange, densely covered with small squamules or tufts which are reddish brown to grey towards the centre and concolorous towards the margin, not translucently striate, dry. Lamellae decurrent, distant, broad, white when young, wax yellow at maturity. Stipe 25–70 × 2–5 mm, cylindrical or tapering downwards, scarlet red to orange red, orange near the base, whitish in the base, glabrous, hollow, dry. Context thin, pale orange red. Smell and taste indistinct. Spore-print white. Basidiospores 9.2–*11*–12.1 × 5.3–*6.1*–6.8 μm, Q = 1.51–*1.81*–2.13, ellipsoid to oblong, often slightly phaseoliform in side view, sometimes with slight median constriction, thin-walled, hyaline. Basidia 4-spored, narrowly clavate. Cystidia absent. Pileipellis at centre and in squamules a trichoderm composed of ascending hyphae constricted at septa, with brown intracellular pigment. Clamp connections present.

Specimen examined—**MONTENEGRO**, **Kolašin Municipality**, Mt. Sinjajevina, Semolj saddle, 42.9092°N 19.2731°E, 1580 m a.s.l., in peat bog, among dense *Sphagnum subsecundum* Nees peat moss with *Carex lasiocarpa* Ehrh. sedge, 18 July 2017, leg. S. Dragićević (NHMP 368/6986; GenBank MN082022).

Ecology & distribution—This waxcap occurs mostly in peat bogs among or near peat moss, but also in wet meadows. Widespread (but generally rare) in Europe, known also from North America and Asia (Tkalčec & al. 2008, Boertmann 2010). In the surrounding countries, *H. coccineocrenata* is recorded in Croatia and Serbia, and it is a strictly protected species in both countries. New to Montenegro.

Comments—*Hygrocybe coccineocrenata* is characterised by decurrent lamellae, predominantly red pileus and stipe (at least in young basidiomata), darker small squamules on the pileal surface, and by living in open, wet habitats (mostly peat bogs). It has often been confused with *H. turunda* (Fr.) P. Karst., which also has darker squamules on pileus, but which differs in its orange to orange yellow pileus and stipe ground colours and growth in rather dry grasslands and heathlands (Boertmann 2010). Our material fits well with the modern descriptions (e.g., Boertmann 2010, Ludwig 2012) of *H. coccineocrenata*. The GenBank BLAST analysis shows that our ITS sequence is identical with one unpublished sequence (FM208899, as "*H. turunda;*" Query Cover 87%), and very close to another sequence from the same authors (FM208865, Identity > 99%, Query Cover 87%; deposited as "*H. cantharellus*," but quite different from the other *H. cantharellus* (Schwein.) Murrill sequences in the database). We assume that both sequences were derived from misidentified samples. Our Montenegrin sequence is the only GenBank entry deposited as *H. coccineocrenata*.

Hypholoma ericaeum (Pers.) Kühner,
Bull. Trimest. Soc. Mycol. Fr. 52: 23 (1936) Fig. 1 c–g

Pileus 17–60 mm broad, paraboloid to hemispherical at first, later convex to plano-convex, often subumbonate or with central papilla, hygrophanous, not translucently striate, brown to dark reddish brown when moist, orange brown to ochre brown on drying, often paler near margin in young basidiomata, surface dry to greasy, often with fine, whitish, fibrillose veil remnants near margin in young basidiomata. Lamellae narrowly adnate, subventricose to ventricose, moderately crowded, broad, pale brownish grey when young, then mottled grey brown, finally mottled dark purplish brown, edge whitish. Stipe 35–78 × 2–7 mm, subcylindrical, often with broadened base (up to 10 mm), solid to fistulose, pale to medium brown, whitish at apex in young basidiomata, surface dry, pruinose at apex, fibrillose-furfuraceous below, mostly with white basal tomentum. Context brown in the narrow zone beneath the pileus surface and above the lamellae, pale brown in the inner part of the pileus, whitish to dark red brown in the stipe. Smell fungoid. Taste slightly bitter. Spore-print violaceous brown. Basidiospores 12.7–*13.7*–15 × 7.5–*8.2*–8.9 µm, Q = 1.56–*1.67*–1.83, ellipsoid, moderately thick-walled to thick-walled, smooth, with distinct and central germ-pore, yellow brown in KOH, light red brown in H_2O. Basidia 4-spored, narrowly clavate or

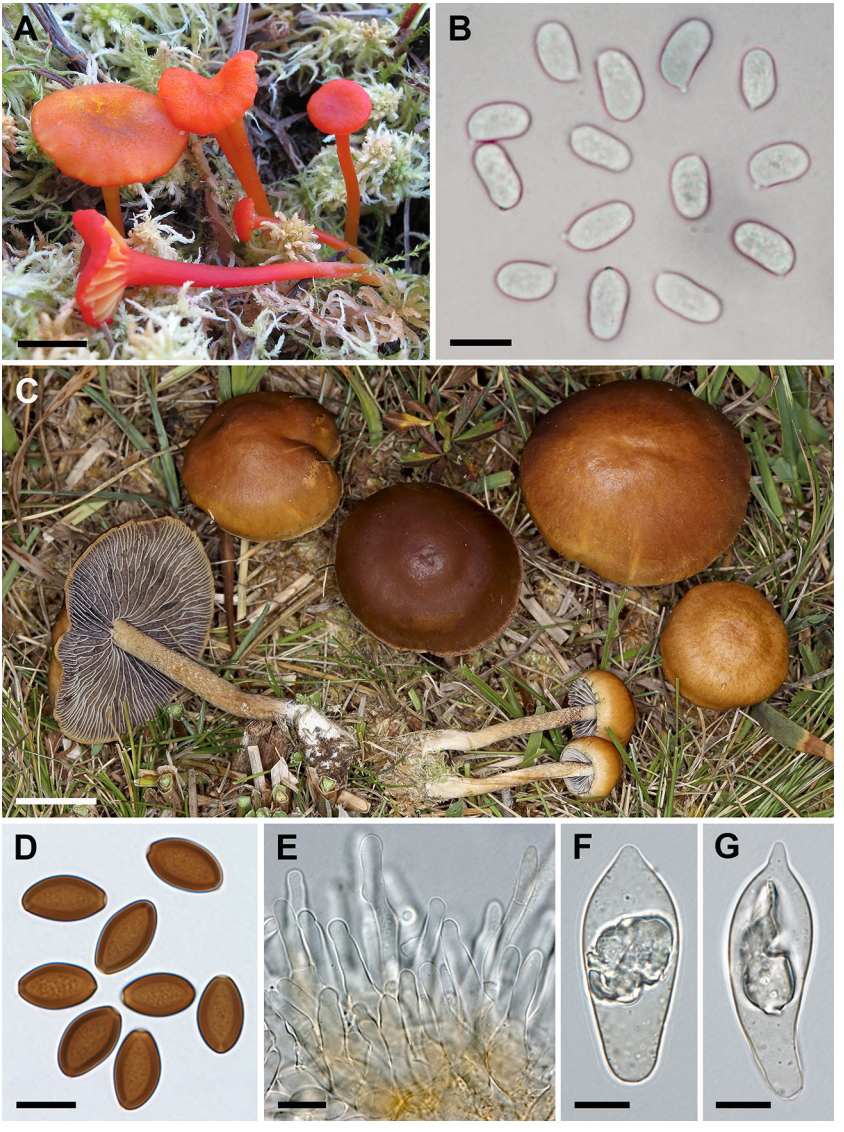

Fig. 1. ***Hygrocybe coccineocrenata*** (NHMP 368/6986). A. basidiomata; B. basidiospores. ***Hypholoma ericaeum*** (NHMP 614/9034). C. basidiomata; D. basidiospores; E. cheilocystidia; F, G. pleurocystidia. Scale bars: A = 10 mm; C = 20 mm; B, D–G = 10 µm.

with median constriction. Cheilocystidia (leptocystidia type) densely packed, forming a sterile lamellar edge, 20–45 × 5–12 µm, lageniform, narrowly utriform or subcylindrical (often with somewhat broadened apex), sometimes forked, thin-walled, hyaline. Pleurocystidia (chrysocystidia type) abundant, 30–56 × 11–18 µm, clavate, mostly with mucronate apex, thin- to moderately thick-walled, hyaline with yellowish amorphous body in alkali solution. Pileipellis a cutis composed of thin-walled, hyaline, repent hyphae. Clamp connections present.

Specimens examined—**MONTENEGRO**, **Kolašin Municipality**, Mt. Sinjajevina, Semolj saddle, 42.9074°N 19.2758°E, 1572 m a.s.l., among *Sphagnum* moss and grass on the edge of the peat bog, 15 September 2018, leg. Z. Tkalčec & al. (CNF 1/7623, NHMP 614/9034; GenBank MN082025); (CNF 1/7628).

Ecology & distribution—The species is saprotrophic and occurs on peaty acid soil (mostly among mosses) and in wet poor grasslands and moist heaths (Watling & Gregory 1987, Noordeloos 2011, Vesterholt & Rald 2012). It is widespread all over the boreal and temperate regions of Europe and North America (Noordeloos 2011), but usually (rather) rare. In the surrounding countries, *H. ericaeum* is recorded only in Croatia (Tkalčec & Mešić 2003). New to Montenegro.

Comments—*Hypholoma ericaeum* is characterised by long basidiospores (>13 µm) and a hygrophanous pileus that is neither translucently striate nor viscid. Only three other European *Hypholoma* species have similarly long basidiospores (*H. eximium* (C. Laest.) Rald, *H. myosotis* (Fr.) M. Lange, and *H. udum* (Pers.) Quél.), but their spore lengths reach at least 17 µm and their pilei are viscid or subviscid. Our collections correspond relatively well with recent descriptions of *H. ericaeum* (Watling & Gregory 1987, Ludwig 2001, Noordeloos 2011, Vesterholt & Rald 2012). However, the maximum stipe width of 7 mm exceeds the 4.5 mm maximum reported by Watling & Gregory (1987), the maximum cheilocystidia width of 12 µm exceeds the 7.5 µm maximum reported by Noordeloos (2011), and the maximum pleurocystidia size of 56 × 18 µm exceeds the 45 × 15 µm maximum reported by Noordeloos (2011). Moreover, Watling & Gregory (1987) described distinctly narrower cheilocystidia (30–40 × 4–5.5 µm) and pleurocystidia (30–40 × 8–10 µm). Noordeloos (2011) described somewhat flattened basidiospores, but the spores in our collections and in the descriptions of other cited authors are not flattened. According to the BLAST analysis, our ITS sequence does not match any sequence in GenBank database.

The closest sequence (96.91% identity) is derived from *H. dispersum* Quél. (HQ604746). The only GenBank ITS sequence annotated as *H. ericaeum* (MH856009) is genetically much more distant (84% identity). It is possible that MH856009 was sequenced from a misidentified sample, but it is also possible that two morphologically close species are not yet recognized as different; further research is required.

Entoloma conferendum (Britzelm.) Noordel.,
Persoonia 10: 446 (1980) Fig. 2 a, b

= *Entoloma nothofagi* G. Stev., Kew. Bull. 16: 234 (1962)

= *Entoloma staurosporum* (Bres.) E. Horak, Sydowia 28: 222 (1976)

Pileus 10–30 mm broad, paraboloid at first, expanding to plano-conical or plano-convex with subumbonate centre, hygrophanous, brown with black centre when young, later greyish brown at centre and gradually fading to light brown towards the margin when moist, pale brownish when dry, translucently striate up to 2/3 of the radius when moist, surface dry, glabrous. Lamellae free, ventricose, broad, moderately crowded, white when young, pink at maturity. Stipe 35–85 × 2–4 mm, cylindrical with slightly to distinctly broadened base, densely silvery striate on pale to light grey brown background, dry. Context thin and fragile, light grey brown. Smell and taste farinaceous. Spore-print dirty pink. Basidiospores 9.3–*10.8*–11.8 × 8.1–*9.4*–10.4 µm, Q = 0.95–*1.15*–1.31, cruciform-stellate, thin-walled, hyaline to pinkish. Basidia 4-spored, narrowly clavate. Cystidia absent. Pileipellis a cutis with transition to a trichoderm at centre, with intracellular brown pigment. Clamp connections absent.

Specimen examined—**MONTENEGRO**, **Rožaje Municipality**, Mt. Hajla, right bank of the Ibar river, 42.7981°N 20.1083°E, 1170 m a.s.l., on dense carpet of peat moss *Sphagnum quinquefarium* (Lindb.) Warnst., on very steep (almost vertical) siliceous rocks, surrounded by mixed forest of spruce (*Picea abies*), fir (*Abies alba*) and beech (*Fagus sylvatica*), 7 September 2017, leg. A. Alegro (NHMP 426/7230; GenBank MN082023).

Ecology & distribution—This species occurs in various habitats from the lowlands to arcto-alpine zone, including grasslands, marshes, peat bogs, damp places in deciduous and coniferous forests. It is a very common saprotroph with a wide distribution, including most temperate zones on both hemispheres (Noordeloos 1992). In the surrounding countries, *E. conferendum* is recorded only in Croatia (Mešić & Tkalčec 2003). New to Montenegro.

Comments—*Entoloma conferendum* is characterised by a brown translucently striate pileus, cruciform-stellate basidiospores (unique among

European *Entoloma* species), and absence of cystidia and clamp connections. Noordeloos & Gates (2012) place it in *E.* sect. *Staurospora* in *E.* subg. *Nolanea* with species having predominantly cuboid basidiospores. *Entoloma staurosporum* is currently accepted as a synonym of *E. conferendum*. Horak (1976, 1980) synonymised *E. nothofagi,* described from New Zealand, with *E. staurosporum*, but subsequently reverted to his original opinion (Horak 1971, 1973), treating *E. nothofagi* as a separate species (Horak 2008). Noordeloos & Gates (2012), who later revised the type of *E. nothofagi*, found only minimal morphological differences from *E. conferendum*, and therefore regarded the two species as probably conspecific. This conclusion is supported by our GenBank BLAST analysis showing the RPB2 sequence of Slovakian collection of *E. conferendum* (KC710191) as almost identical with the RPB2 sequence of *E. nothofagi* from New Zealand (MH190134), with only a single base pair difference between them. There is no ITS sequence available for *E. nothofagi*. Another similar species is *E. brevispermum* G.M. Gates & Noordel. from Australia, which differs by shorter (≤9 µm long) basidiospores. Our material fits well with the modern descriptions of the species (Noordeloos 1992, Ludwig 2007, Noordeloos & Gates 2012), and our ITS sequence matched well (identity > 99%) with most *E. conferendum* sequences in GenBank.

Bovista paludosa Lév., Ann. Sci. Nat., Bot., Sér. 3, 5: 163 (1846) Fig. 2 c, d

Basidiomata pyriform, 23–48 mm high and 16–24 mm broad, sometimes with white rhizoids. Exoperidium when young white and smooth or with small, very thin, adherent, greyish, polygonal patches, later breaking up into irregular, rather large plates. Endoperidium smooth, white at first, becoming brown to olivaceous. Gleba white when young, then yellowish brown, becoming olive brown at maturity. Subgleba well developed, ≤15 mm high, compact, white when young, olive to grey brown at maturity. Spore-print olive brown. Basidiospores 4.6–*5.1*–5.6 × 4.2–*4.7*–5.4 µm, Q = 1–*1.07*–1.19, globose to subglobose (rarely broadly ellipsoid), almost smooth to asperulate, moderately thick-walled, pale yellow brown in KOH, with sterigmal remnant ≤15 µm long. Capillitium Bovista-type, non-poroid, thick-walled, rarely septate.

Specimen examined—**MONTENEGRO, Žabljak Municipality, Mt. Durmitor,** Barno jezero lake, 43.1582° N, 19.0932° E, 1495 m a.s.l., among *Aulacomnium palustre* (Hedw.) Schwägr., *Bryum pseudotriquetrum* (Hedw.) P. Gaertn. & al., and *Campylium stellatum* (Hedw.) C.E.O. Jensen mosses on the outer zone of the peat bog (less acidic), 9 July 2017, leg. I. Ćetković (NHMP 425/7229; GenBank MN082024).

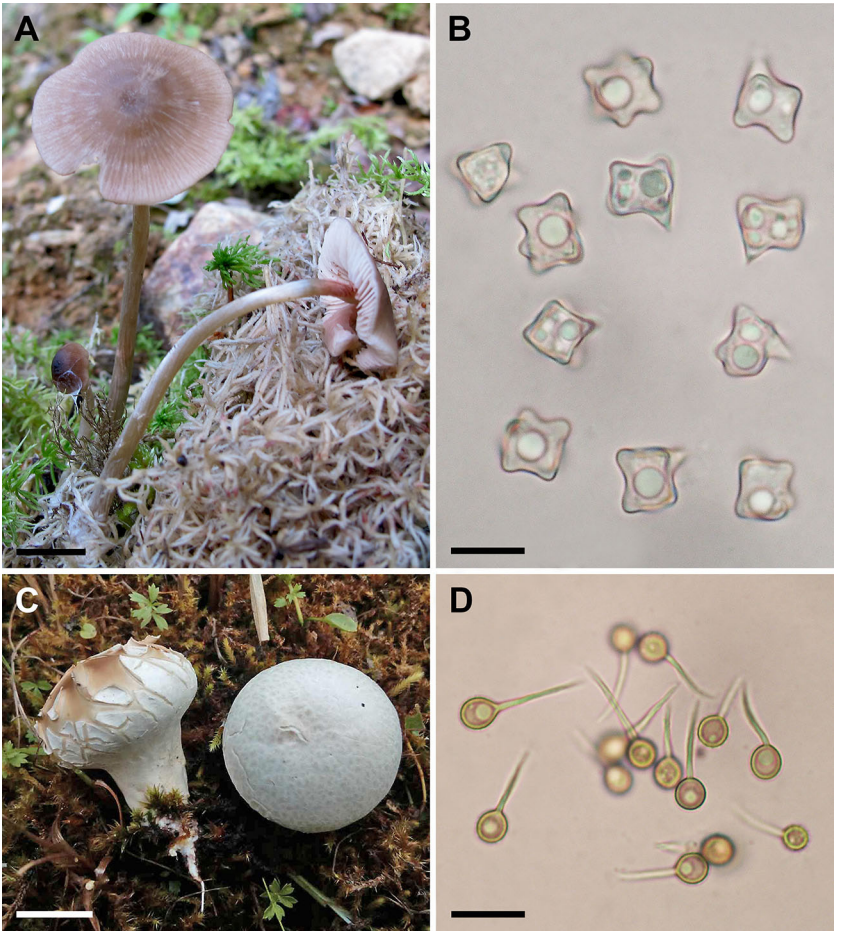

Fig. 2. ***Entoloma conferendum*** (NHMP 426/7230). A. basidiomata; B. basidiospores. ***Bovista paludosa*** (NHMP 425/7229). C. basidiomata; D. basidiospores. Scale bars: A, C = 10 mm; B, D = 10 μm.

Ecology & distribution—This puffball lives as a saprotroph in a rather wide range of open wet mossy habitats, most often in alkaline to neutral fens, bogs, mires, wet meadows, and heaths, but avoiding oligotrophic and strongly acidic mires (Kałucka 2019). The species is rather widespread but rare in Europe (Red Listed in most countries where reported), known also from Asia (China, Russia, India) and North America (Canada) (Jeppson 2018). The only earlier

Montenegrin record of *B. paludosa* from 1904 (Bubák 1915) was published without locality and habitat data. In the surrounding countries, it is recorded only in Serbia (Kałucka 2019).

Comments—*Bovista paludosa* is characterised by its occurrence on wet mossy habitats and by the presence of a well-developed subgleba, long sterigmal remnants on basidiospores, and a Bovista-type capillitium lacking pores. The most similar species, *B. cretacea* T.C.E. Fr., differs in a very poorly developed (≤2 mm high) subgleba and regularly septate capillitium. Our material agrees with the modern descriptions (Pegler & al. 1995, Sarasini 2005, Jeppson 2018). According to the BLAST analysis, our ITS sequence matched well with all three sequences of *B. paludosa* in GenBank database (Identity > 99%).

Acknowledgements

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